

Award Number: W81XWH-08-1-0660

TITLE: TPD52: A Novel Vaccine Target for Prostate Cancer

PRINCIPAL INVESTIGATOR: Robert K. Bright, Ph.D.; Jennifer D. (Lewis) Bright, M.S. (Associate)

CONTRACTING ORGANIZATION: Texas Tech University Health Science Center, Lubbock, Texas, 79430-6591

REPORT DATE: September 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

✓ Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

| REPORT DOCUMENTATION PAGE | | | Form Approved OMB No. 0704-0188 | |
|---|------------------|--------------------------|---|---|
| Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS. | | | | |
| 1. REPORT DATE (DD-MM-YYYY) 01-09-2010 | | 2. REPORT TYPE Annual | | 3. DATES COVERED (From - To) 1 Sept 2009-31 Aug 2010 |
| 4. TITLE AND SUBTITLE TPD52: A Novel Vaccine Target for Prostate Cancer | | | 5a. CONTRACT NUMBER | |
| | | | 5b. GRANT NUMBER W81XWH-08-1-0660 | |
| | | | 5c. PROGRAM ELEMENT NUMBER | |
| 6. AUTHOR(S) Robert K. Bright, Ph.D.; Jennifer D. (Lewis) Bright, M.S. email: robert.bright@ttuhsc.edu | | | 5d. PROJECT NUMBER | |
| | | | 5e. TASK NUMBER | |
| | | | 5f. WORK UNIT NUMBER | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Texas Tech University Health Sciences Center, Lubbock, Texas, 79430-6591 | | | 8. PERFORMING ORGANIZATION REPORT NUMBER | |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012 | | | 10. SPONSOR/MONITOR'S ACRONYM(S) | |
| | | | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) | |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited | | | | |
| 13. SUPPLEMENTARY NOTES | | | | |
| 14. ABSTRACT The overall goal of this Award is to test the efficacy of TPD52-based vaccines in the TRAMP murine model of prostate cancer, and to characterize vaccine induced mechanisms of tumor immunity. We have continued our evaluation of the ability of TPD52-DNA and/or TPD52-protein based vaccines to induce immune responses capable of rejecting the formation of subcutaneous tumors following challenge with prostate-derived TRAMP-C1 or TRAMP-C2 tumor cells. Over the past 12 months we have made the following significant findings or accomplishments; First, DNA vaccines encoding the human TPD25 (hD52) induced increased protection from not only primary challenge, but secondary challenge with a distinct TRAMP-C tumor, compared to murine TPD52 (mD52)-DNA based vaccines suggesting the hD52 xeno-antigen was capable of inducing more effective memory responses. Second, the cytokine profile from vaccine induced T cells indicated a cellular Th1-type response that was important for tumor protection. Third, in vivo inhibition of TGFβ-1 with TPD52-protein vaccination increased protection from tumor challenge demonstrating a regulatory role for TGFβ-1. Finally, T cell cytokine analyses revealed the presence of a population of CD8+ MHC-I-restricted T cells that secrete IL-10 in an antigen-specific manner, suggesting a novel role for CD8+ Treg cells in suppressing TPD52-based vaccination in our model. | | | | |
| 15. SUBJECT TERMS Human TPD52 (hD52); murine TPD52 (mD52); transgenic adenocarcinoma mouse prostate (TRAMP); xenogeneic antigen; T regulatory cells (Treg); transforming growth factor beta 1 (TGF-β1) | | | | |
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT UU | 18. NUMBER OF PAGES 13 |
| a. REPORT U | b. ABSTRACT U | c. THIS PAGE U | | |
| | | | | 19a. NAME OF RESPONSIBLE PERSON USAMRMC |
| | | | | 19b. TELEPHONE NUMBER (include area code) |

Table of Contents

| | <u>Page</u> |
|-----------------------------------|-------------|
| Introduction..... | 4 |
| Body..... | 5 |
| Key Research Accomplishments..... | 10 |
| Reportable Outcomes..... | 10 |
| Conclusion..... | 12 |
| References..... | 13 |
| Appendices..... | NA |

INTRODUCTION

Recently the National Cancer Institute sponsored a pilot project to prioritize cancer vaccine target antigens for translational research. The study involved developing a set of nine ideal cancer antigen criteria/characteristics, with therapeutic function, immunogenicity and a role in oncogenicity deemed as having the greatest weight or importance. From a representative list of antigens, a group of antigens defined as over-expressed self-proteins stands out as the group with the largest number of candidate target antigens [1]. It is not clear what modulation measures are necessary for breaking tolerance to over-expressed self-antigens. Lack of knowledge in this area is an important problem that will inhibit development of vaccines for many life-threatening cancers including prostate cancer.

Tumor protein D52 (D52) is a novel over-expressed self-protein involved in cellular transformation, proliferation and metastasis. D52 over-expression has been demonstrated in several human malignancies including prostate [2, 3, 4], breast [5, 6, 7, 8], and ovarian [9] carcinomas. Recent studies have identified TPD52 as one of 12 important markers, along with MUC-1 and PSA, that can be used as a molecular fingerprint of human prostate cancer enabling more accurate and sensitive diagnosis and prognosis of aggressive disease [10]. Our laboratory independently identified and cloned TPD52 from human prostate cancer cells, isolated from patients undergoing radical prostatectomy, using differential gene expression analysis of our novel paired cancer and normal human prostate epithelial cell cultures. The murine orthologue of *TPD52* (*mD52*) naturally mirrors *hD52* with respect to known function and over-expression in tumor cells, and shares ~86% protein identity with the human orthologue [11]. Recently we demonstrated that transfection and stable expression of *mD52* cDNA in mouse 3T3 fibroblasts (3T3.mD52) induced increased proliferation, anchorage independent cell growth, and the ability to form subcutaneous tumors and spontaneous lethal lung metastases *in vivo* when 3T3.mD52 cells were inoculated subcutaneously into naïve, syngeneic, immune-competent mice [12]. Together, these data strongly suggest that *TPD52* expression may be important for initiating and perhaps maintaining a tumorigenic and metastatic phenotype and thus may be important for tumor cell survival. Thus, D52 represents a non-mutated over-expressed self-oncoantigen. We demonstrated for the first time that mD52 induces protection against tumor challenge when administered as recombinant protein-based vaccine with CpG-ODN [13]. We also reported that mD52 DNA vaccination induced an immune response that rejected tumors in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model of prostate cancer. The T cell cytokine secretion patterns indicated that a T_H-1-type cellular immune response was involved [14]. Others reported that an mD52 overlapping peptide vaccine was effective in a murine breast cancer model [15].

Our goal is to determine the best modulation measures needed with vaccination to effectively target TPD52. The Transgenic Adeno-carcinoma of the Mouse Prostate (TRAMP) model was employed to study TPD52 as a vaccine antigen. Naïve mice were immunized with either mD52 or hD52 plasmid DNA or recombinant protein. In some experiments, modulation of regulatory T cells or TGF- β 1 accompanied vaccination. Following immunization, mice were challenged with a tumorigenic dose of mD52 positive, autochthonous TRAMP tumor cells. Significant tumor protection was observed more than ten months post tumor challenge. In some experiments survivors of initial tumor challenge rejected a second tumor challenge with TRAMP tumor cells given approximately one hundred and forty days after the first challenge and remained tumor free for more than six months. The T cell cytokine secretion patterns from tumor challenge survivors indicated that a cellular immune response was involved in tumor rejection, and that a unique subset of regulatory CD8 T cells may play a role in inhibiting vaccine induced tumor immunity. These data demonstrate that TPD52-vaccination induces a memory, cellular immune response capable of providing protection from tumor challenge with murine prostate-derived tumor cells naturally over-expressing the mD52 self-oncoantigen. The data also suggest that a deeper understanding of the role of regulatory CD8 T cells in inhibiting TPD52 vaccine-induced immunity may lead to a more effective vaccine.

BODY

The following research accomplishments reflect the period from September 1, 2009 through August 31, 2010. During this funding period we extended our studies on DNA-based vaccines to include assessment of memory responses elicited by xenogeneic DNA immunizations compared to those induced by the murine orthologue of TPD52. Evaluation of T cell responses to TPD52 following vaccination, and evaluation of peripheral mechanisms of immune regulation of responses against the over-expressed self-oncoantigen TPD52 were examined. In addition, we began studies to assess the efficacy of xenogenic hD52 protein and DNA/protein heterologous prime boost to protect against tumor challenge. Concurrent with these new vaccine studies were studies addressing the role of TGF- β 1 and T regulatory cells in suppressing vaccine induced immunity as well as studies to examine the role different T cell subsets play in TPD52 vaccine induced immunity *in vivo*.

Research Accomplishments

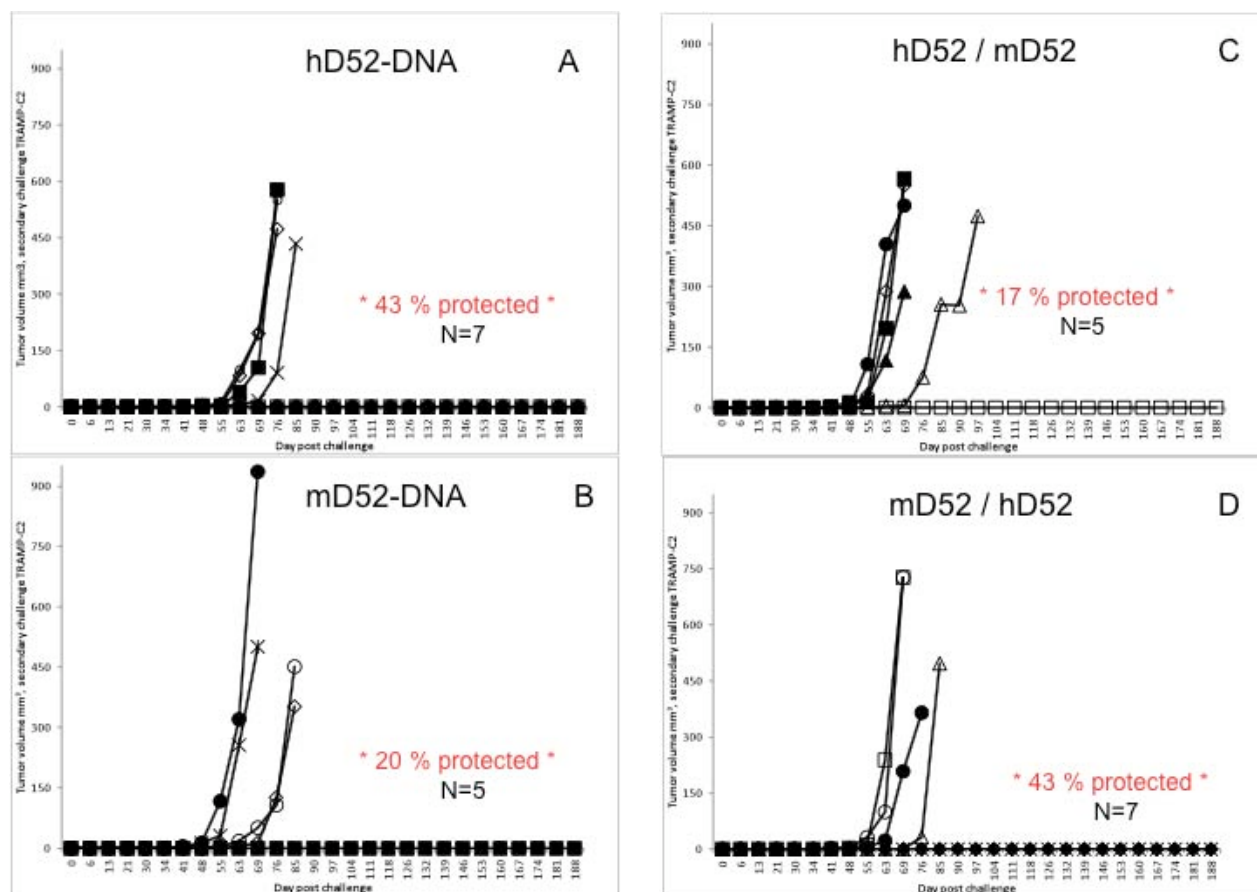


Figure 1. Immunization with TPD52 DNA induces protection against secondary recurrent tumor challenge. Groups of male C57BL/6 mice were immunized with 50 micrograms of TPD52-DNA administered i.m. in saline every 10 days for a total of 4 injections. Two weeks after the 4th injection the mice were challenged s.c. with TRAMP-C1. Six months following rejection of primary challenge mice were challenged with TRAMP-C2 tumor cells in the opposite flank. **A)** Mice were immunized with human TPD52 (hD52)-DNA and challenged with 1×10^6 autochthonous TRAMP-C2 tumor cells 180 days after primary challenge. **B)** Mice were immunized with murine TPD52 (mD52)-DNA then challenged with 1×10^6 autochthonous TRAMP-C2 tumor cells 180 days after primary challenge. **C)** Mice were immunized with hD52-DNA twice followed by mD52-DNA twice then challenged with 1×10^6 autochthonous TRAMP-C2 tumor cells. **D)** Mice were immunized with mD52-DNA twice followed by hD52-DNA twice then challenged with 1×10^6 autochthonous TRAMP-C2 tumor cells. Tumor size was determined by taking perpendicular measurements with calipers every 2 to 3 days and tumor volume (mm^3) was calculated using the following formula: $(a \times b^2) / 2$, where b is the smaller of the two measurements.

For our continued efforts to address the induction of immunologic memory following intramuscular administration of TPD52-DNA as a vaccine we immunized groups of male C57BL/6 mice with plasmid DNA encoding either murine TPD52 (mD52) or human TPD52 (hD52) as a xenogenic tumor associated antigen, or combination of hD52 and mD52 as a heterogeneous prime-boost approach. Following immunization, we tested whether vaccination was capable of providing long-term immunity against recurrence modeled by secondary challenge. We demonstrated previously that the ~15% difference in amino acid content between hD52 and mD52 imparted greater immunogenicity to hD52 in mice and apparent intramolecular epitope spreading resulting in immunity to the native murine form of TPD52 expressed by murine tumor cells resulting in their rejection. Seventy percent of mice (7/10) immunized with hD52-DNA were capable of long term rejection of primary TRAMP-C1 tumor cell challenge whereas only 50% of mice (5/10) immunized with mD52-DNA were protected from primary TRAMP-C1 tumor cell challenge (see progress report 2009). Greater than 40% of the primary challenge survivor mice immunized with either hD52-DNA alone or mD52-DNA prime and hD52-DNA boost survived a secondary challenge with TRAMP-C2 cells in the opposite flank ~six months after primary challenge/ rejection (Fig. 1A, D). This was superior to what was observed for mD52-DNA alone and hD52-DNA prime followed by mD52-boost (Fig. 1B, C). This supports the hypothesis that xenogeneic hD52 is more immunogenic than mD52, particularly if administered alone or as a vaccine boost. Though we continue to work toward complete protection from tumor challenge we were encouraged by the results with hD52-DNA as a xenogenic antigen-based vaccine.

Analysis of cytokine production by tumor-specific T cells from hD52-DNA immunized mice revealed production of IFN- γ (Fig. 2A), but no IL-4, IL-17 or TGF- β 1 was detected (Table 1) indicating induction of a T_H1 -type immune response. Of note, a second population of CD8+ IL-10 producing T cells was uncovered (Fig. 2B). These CD8+ cells were MHC-I-restricted, mD52-specific evidenced by recognition of TRAMP-C1 and TRAMP-C2 tumor cells but not Yac-1 or mKSA (Balb/c), and by the ability of anti-MHC-I mAb to inhibit tumor recognition and subsequent IL-10 production. These data provide important support for our hypothesis that CD8+ CD122+ Tregs and IL-10 may suppress TPD52-specific vaccine-induced immune responses along with CD4+ CD25+ Tregs.

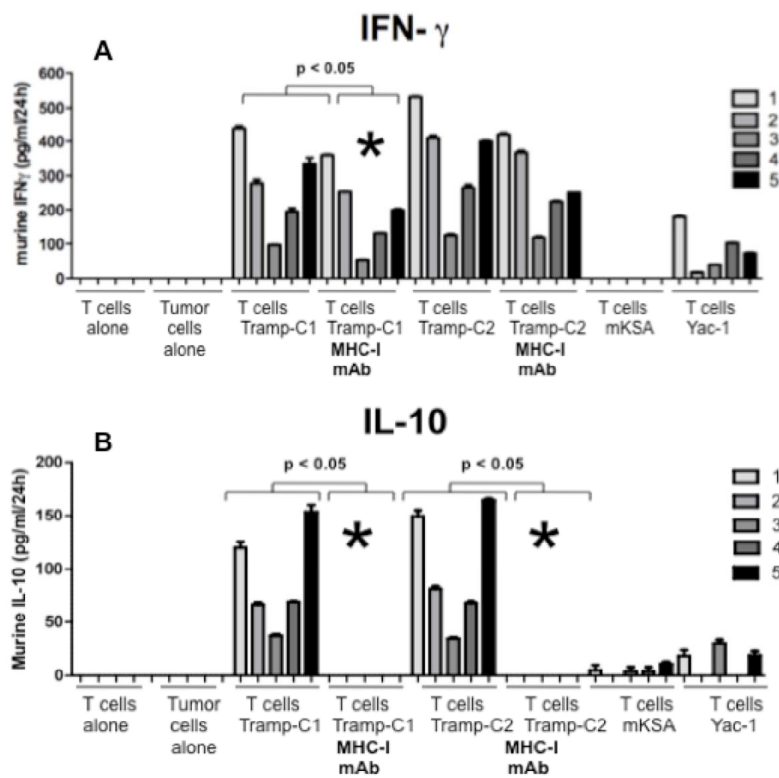


Figure 2. hD52-DNA vaccine induces IFN- γ and uncovers CD8+ IL-10+ Treg cells. The data show IL-10 and IFN- γ production by T cells as picograms per ml of culture medium from 24h incubations. The gray and black bars represent 5 individual mice in equal groups of 5, cultured alone or with various targets. Targets include: TRAMP-C1 and -C2 (mD52+, MHC-I-matched) and mKSA (mD52+, MHC-I-mis-matched). The asterisk denotes inhibition of IL-10 or IFN- γ production by anti-H2-b mAb. Methods: Male C57BL/6 mice were immunized, tumor challenged, and tumor growth determined as described in the legend of Figure 1. T cell culture and analysis of cytokine production were performed using our previously published methods [13, 14]. Values = mean \pm SEM for replicates. Significance: one-way ANOVA with Bonferroni's post-test.

As stated previously we planned to extend our TPD52 vaccine studies to include TPD52-protein based vaccines. The full-length cDNAs for mD52 and hD52 were sub-cloned separately into the pGEX-3X bacterial expression vector containing glutathione S-transferase (GST) as a purification tag and carrier protein. Both mD52-GST and hD52-GST fusion proteins have a molecular weight of approximately twice that of each protein alone (~50kD) due to inclusion of the GST carrier protein. We demonstrated the ability to generate purified mD52 protein previously (see progress report for 2009). We are now able to easily generate >10 mg of highly purified hD52 protein for our vaccines studies (Fig. 3). We have demonstrated the efficacy of using GST as a carrier protein to increase the immunogenicity of self or auto-antigens when administered as vaccines in murine tumor models [13]. We used mD52 and hD52 protein in the following vaccine studies to begin to address the role of CD4+ CD25+ Tregs or TGF- β 1 in suppressing TPD52 vaccine induced tumor immunity.

As alluded to above a mechanism possibly contributing to the lack of complete protection is related to the normal cell expression of TPD52 and CD4+ CD25+ Treg suppression of immunity to self-proteins. TPD52 is a non-mutated, tumor associated antigen that is expressed by several normal tissues at levels less than observed for malignant cells [13, 14]. This expression pattern makes TPD52, by definition, a tumor-associated auto-antigen and subject to peripheral mechanisms of immune suppression and regulation, most notable the action of CD4+ CD25+ Foxp3+ regulatory T cells (Tregs). To address this possibility, we depleted CD25+ cells *in vivo* along with TPD52-protein vaccination by administration of PC61 monoclonal Ab known to effectively target and deplete CD25+ T regs in mice.

Groups of mice were immunized s.c. with TPD52 protein in IFA every ten days, for a total of four injections prior to challenge with $\sim 5 \times 10^5$ TRAMP-C2 tumor cells fourteen days following the final immunization. The vaccine consisted of either 50 μ g of mD52 protein or 50 μ g of hD52 protein both mixed with 50 μ g of CpG/ODN [13]. Controls were IFA alone, IFA and CpG/ODN. We published previously that vaccines comprised of TPD52 protein without CpG/ODN were not immunogenic [13, 14]. Some groups of mice were depleted of CD25+ Tregs along with vaccination by injection (i.p.) of the anti-CD25 mAb PC61 (for method details see the legend of figure 4). Similar to control immunized mice (cntrl bar), none of the mice immunized with either mD52 or hD52 protein with CpG/ODN in IFA (mock

| Cytokine production | Vaccine | | MHC-I mAb Blocking |
|---------------------|--------------|----------------------------|--------------------|
| | hD52 DNA x 4 | mD52 DNA x 2/ hD52 DNA x 2 | |
| IL-4 | - | - | N/A |
| IL-17 | - | - | N/A |
| TGF- β 1 | - | - | N/A |
| IL-10 | + | + | + |
| IFN- γ | + | + | + |

Table 1. Cytokine Profile of Mice Surviving Tumor Challenge with TRAMP-C1 and Re-challenge with TRAMP-C2. Splenocytes were harvested from surviving mice and incubated with irradiated TRAMP tumor cells. Supernatants were collected after 24 hrs and assayed for IL-17, IL-4, TGF- β 1, IL-10, or IFN- γ (1:4 for all cytokines except IFN- γ ; 1:120). TRAMP-C1 and TRAMP-C2 are mD52 expressing MHC matched tumors, mKSA is mD52 expressing, MHC mis-matched tumor cell, Yac-1 is a control for non-specific T-cell activity. In separate conditions an MHC-I H-2b blocking monoclonal antibody was included with TRAMP-C1 and TRAMP-C2 as denoted by TRAMP-C1 + mAb and TRAMP-C2 + mAb.



Figure 3. Western blot showing purification of hD52 protein: Lane 1 is a molecular weight ladder, lane 2 is skipped, lane 3 is a sample post lysis of *E. coli* cells expressing hD52 protein, lane 4 is flow through, lane 5 is wash 1, lane 6 is wash 2, lane 7 is wash 3, lane 8 is wash 4, lane 9 is wash 5, lane 10 is eluate 1, lane 11 is eluate 2, lane 12 is eluate 3, lane 13 is eluate 4. Lanes 10-13 represent purified hD52-GST fusion protein. To detect hD52 protein expression 1 μ g of anti-TPD52 antibody was added.

depleted bar) were capable of rejecting tumor challenge as 100% (10/10) of the animals were bearing large tumors by day 55 post tumor challenge (Fig. 4A, B). Lack of the ability to induce tumor immunity with this vaccine approach in the absence of CD25 Treg cell depletion was also observed in two distinct murine strains with two distinct tumor types. We postulate that this may be a result of the tightly regulated immunologic environment in the s.c. region compared to the i.m. route or region. This is supported by the fact that this method of vaccination was highly effective if accompanied by modulation of immune regulation in the form of CD25+ Treg cell depletion. Only 4/10 mice and 2/10 mice developed tumors when depleted of CD25+ Tregs if concurrently immunized with mD52 protein and CpG/ODN or hD52 protein and CpG/ODN, respectively (Fig. 4A, B) (CD25 depleted bar). These data indicate that peripheral mechanisms of cellular immune suppression are actively inhibiting vaccine-induced cellular immune responses to the over-expressed self-oncoantigen TPD52. These findings are promising in that they demonstrate that whole protein TPD52 vaccine may be developed as a vaccine approach for prostate cancer if the vaccine includes strategies to inhibit Treg mediated immune suppression. Flow cytometric analysis to monitor CD25 Treg cell depletion in the peripheral blood was performed throughout the experiment and indicated that we obtained ~70% and 40% depletion of CD25 cells in mD52 and hD52 protein immunized mice, respectively (data not shown). Greater depletion of

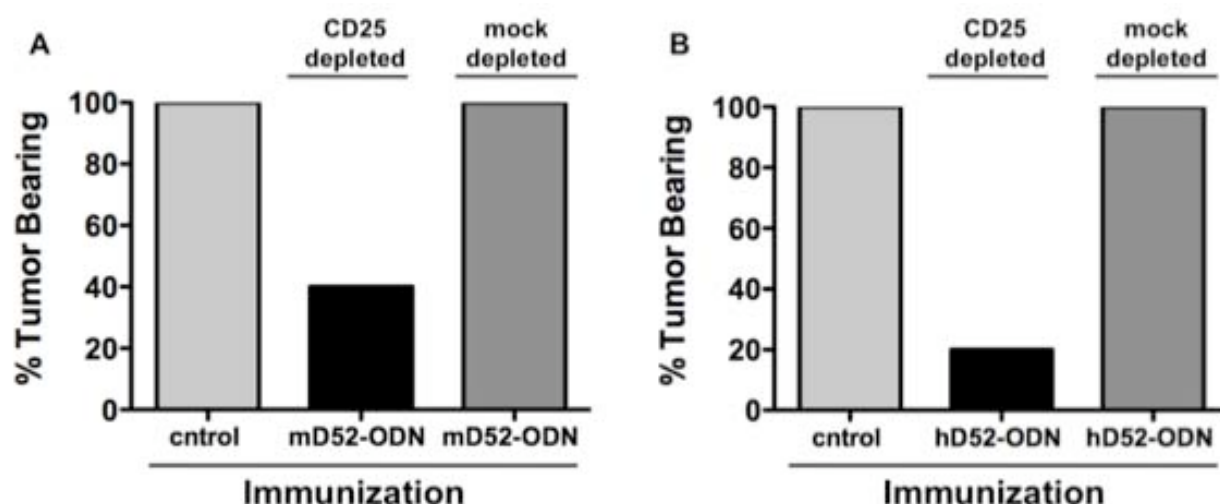


Figure 4. TPD52-protein vaccination is enhanced by CD25 Treg depletion. The data show the percent of mice bearing tumors on day 55 post tumor inoculation following four immunizations with recombinant TPD52-protein + CpG-ODN in IFA with mock depletion (right bar) or in vivo CD25 depletion (center bar). The vaccine was ineffective unless administered with anti-CD25 mAb, indicating that TPD52-protein vaccines require systemic depletion of CD25 Tregs to be effective. **Methods:** Male C57BL/6 mice were immunized with **A)** mD52 protein or **B)** hD52 protein as described in text. Fourteen days following the final immunization individual mice were challenged (s.c.) with $\sim 5 \times 10^5$ autochthonous TRAMP-C2 tumor cells in PBS. CD25 Treg depletion was accomplished by injection (i.p.) of 300 μ g of the specific mAb PC-61 with the first and third immunizations and 600 μ g just prior to tumor challenge for a total of 3 injections. Tumor size was determined as detailed in the legend of Figure 1. Shown are representative data for repeated experiments.

CD25 cells in mD52 protein immunized mice resulted in ~50% greater tumor burden in that group of mice compared to mice immunized with hD52 protein and depleted of CD25 cells. Since effector T cells also express CD25, the high affinity α subunit of the IL-2 receptor, these results suggest that greater than 50% depletion of CD25 cells may also inhibit T cell effector cell function and subsequent tumor immunity. This will be examined further in the months to come.

It is widely accepted that TGF- β 1 acts on peripheral T cells to drive them to a regulatory function, known as inducible Tregs cells. It has been demonstrated that tumor cells often secrete significant quantities of TGF- β 1 into the tumor microenvironment. We confirmed that both TRAMP-C1 and TRAMP-C2 tumor cells secrete nearly 1,000 pico grams of TGF- β 1 per ml of culture medium per 24hr (data not shown). This amount of secreted TGF- β 1 was comparable to that of other murine and human tumor cell lines. This observation lead us to hypothesize that *in vivo* inhibition of TGF- β 1 could

have a similar effect, as we observed with CD25 Treg cell depletion, on enhancing tumor immunity following TPD52 vaccination.

To address this, groups of mice were immunized s.c. with TPD52 protein in IFA every ten days, for a total of four injections prior to challenge with $\sim 5 \times 10^5$ TRAMP-C2 tumor cells fourteen days following the final immunization. The vaccine consisted of either 50 μ g of mD52 protein or 50 μ g of hD52 protein both mixed with 50 μ g of CpG/ODN [13]. Controls were IFA alone, IFA and CpG/ODN. We published previously that vaccines comprised of TPD52 protein without CpG/ODN were not immunogenic [13, 14]. Some groups of mice were depleted of TGF- β 1 along with vaccination by injection (i.p.) of the anti-TGF- β 1 mAb 1D11 (for method details see the legend of figure 5). Similar to control immunized mice (ctrl bar) and as we observed for CD25 depletion experiments in figure 4, none of the mice immunized with either mD52 or hD52 protein with CpG/ODN in IFA (mock depleted bar) were capable of rejecting tumor challenge as 100% (10/10) of the animals were bearing large tumors by day 55 post tumor challenge (Fig. 5A, B). However, significant anti-tumor immunity was observed if TPD52 protein vaccination was accompanied by *in vivo* TGF- β 1 inhibition. Only 4/10 mice and 2/10 mice developed tumors when TGF- β 1 was inhibited in combination with mD52 protein and CpG/ODN or hD52 protein and CpG/ODN immunization, respectively (Fig. 5A, B) (TGF- β 1 depleted bar). These data further support our hypothesis and observations that peripheral mechanisms of cellular immune suppression are actively inhibiting vaccine-induced cellular immune responses to the over-expressed self-oncoantigen TPD52. Again, these findings are promising in that they demonstrate that whole protein TPD52 vaccine may be developed as a vaccine approach for prostate cancer if the vaccine includes strategies to inhibit Treg mediated immune suppression, in this example prior to Treg induction, by upstream blocking of TGF- β 1. It was not possible to monitor TGF- β 1 blocking *in vivo* by flow cytometric analysis in the peripheral blood. To this end, it is not clear why there appears to be a 50% difference in tumor protection between mD52 protein and hD52 protein immunized mice, respectively. This difference will be further examined in future experiments.

In our current ongoing experiments, we have begun to assess the efficacy of heterologous DNA-prime protein-boost approaches for vaccinating to induce tumor immunity. In addition, we have ongoing experiments to assess the role of T cell subsets *in vivo* by targeted depletion with vaccination. These studies will be reported on in our 2011 progress report.

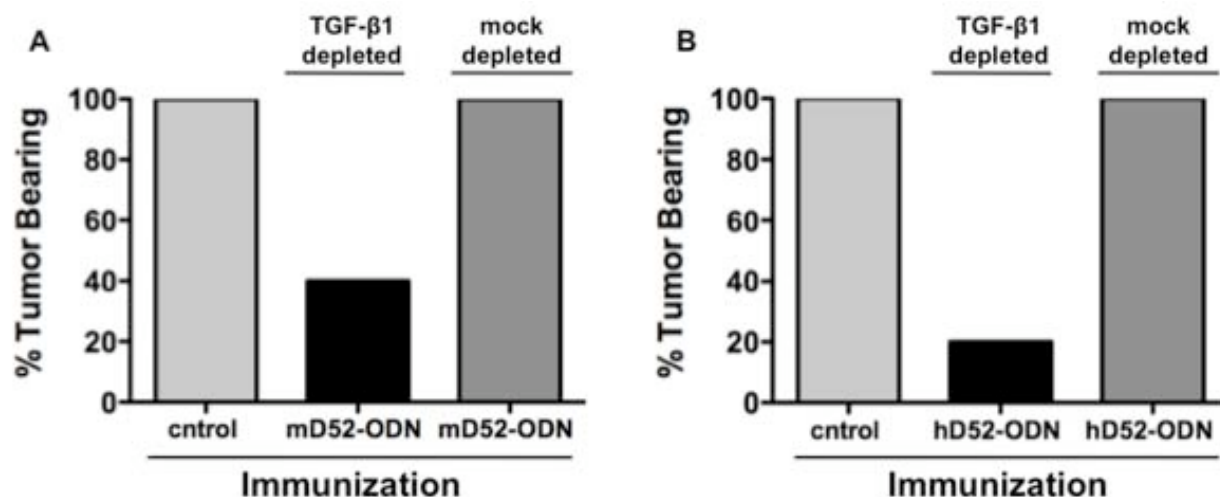


Figure 5. TPD52-protein vaccination is enhanced by TGF- β 1 inhibition. The data show the percent of mice bearing tumors on day 55 post tumor inoculation following four immunizations with recombinant mD52-protein + CpG-ODN in IFA with mock depletion (right bar) or TGF- β 1 depletion (center bar). The vaccine was ineffective unless administered with anti-TGF- β 1 mAb, indicating that TPD52-protein vaccines require systemic inhibition of TGF- β 1 to be effective. Methods: Male C57BL/6 mice were immunized with **A)** mD52 protein or **B)** hD52 protein as described in the text. Fourteen days following the final immunization individual mice were challenged (s.c.) with $\sim 5 \times 10^5$ autochthonous TRAMP-C2 tumor cells in PBS. TGF- β 1 inhibition was accomplished by injection (i.p.) of 100 μ g of the specific mAb 1D11 beginning one day prior to tumor challenge and every three days following challenge for a total of 12 injections. Tumor size was determined as detailed in the legend of Figure 1. Shown are representative data for repeated experiments.

Future Work

In the coming weeks and months we will perform T cell function assays and determine tumor volume on mice primed with hD52-DNA and boosted with mD52-protein. In addition, we will have data on the role of CD4 and CD8 T cells *in vivo* from our ongoing experiments to deplete these T cell subsets with hD52-DNA prime mD52-protein boost vaccination. We will perform EliSpot assays for IFN- γ production, ELISA based cytokine capture assays for the production of IL-4, IL-10, IL-17 and IFN- γ to assess the role of various T Helper cell subsets, and CTL-mediated killing assays to demonstrate T cell effector function against tumor cell targets. For the upcoming year, we will further evaluate and optimize DNA prime protein boost vaccine strategies for the ability to induce complete protective tumor immunity. We will also determine a role for regulatory T cells both CD4+ and CD8+ by administering antibodies to CD25 (CD4 Treg) and/or CD122 (CD8 Treg), as well as antibodies to TGF β -1 to assess their roles in suppression of tumor immunity following TPD52-based vaccination.

KEY RESEARCH ACCOMPLISHMENTS

(The research accomplishments outlined below were completed by a 50% FTE associate, a graduate student and myself at 30% effort)

- We demonstrated that the human orthologue of TPD52 (hD52), delivered as a DNA-based vaccine, is more effective at inducing protective tumor immunity than murine TPD52 (mD52) in the TRAMP model of prostate cancer.
- We demonstrated that hD52-DNA-based vaccine is more effective at inducing immunologic memory and subsequent protective tumor immunity against recall tumor challenge than mD52 in the TRAMP model of prostate cancer.
- We demonstrated that TPD52-DNA vaccination induces a Th1-type cellular immune response that plays an important role in TPD52 vaccine induced tumor immunity.
- We successfully generated large quantities of recombinant hD52 protein for vaccine studies.
- We demonstrated that CD25+ Tregs play a role in suppression of effective tumor immunity following TPD52 protein-based vaccination.
- We demonstrated that TGF β -1 plays a role in suppression of effective tumor immunity following TPD52 protein-based vaccination.
- We uncovered a novel population of CD8+ IL-10 secreting, antigen-specific, MHC-I-restricted Treg cells that may be a critical cellular component, along with CD4+ CD25+ Tregs, involved in suppressing vaccine induced immunity to TPD52.
- We initiated studies to evaluate the efficacy of heterologous, DNA-prime protein-boost vaccine strategies.

REPORTABLE OUTCOMES

Abstracts:

- AACR annual meeting accepted 1/26/2010: "Inhibition of Tumor Formation in Mice following Vaccination with DNA Encoding a Novel Xenogeneic Tumor Associated Auto-Antigen". Jennifer D. Bright, Heather N. Schultz, and Robert K. Bright, Departments of Microbiology & Immunology and Urology, Texas Tech University Health Sciences Center, Lubbock, Texas
- ImPact meeting, accepted 8/26/2010: "Prostate Cancer Vaccines Targeting Over-Expressed Self-Oncoantigens". Robert K. Bright, Texas Tech University Health Sciences Center; Jennifer D. Bright, Texas Tech University Health Sciences Center; Heather N. Schultz, University of Texas at Austin ; Jennifer A. Byrne, The University of Sydney Discipline of Paediatrics and Child Health, Childrens Hospital at Westmead, NSW, Australia.

Manuscripts:

- 1) Bright JD, Schultz HN, Byrne JA and **Bright RK**. Peripheral regulation of a DNA vaccine targeting a prostate-associated self-oncoantigen (submitted)
- 2) Bright JD, Schultz HN, Byrne JA and **Bright RK**. Combination Immuno-Modulation Strategy to Enhance Immunity to the Tumor Associated Auto-Antigen TPD52 (in preparation).
- 3) Wolfe AR, Bright JD, Moghaddam MZ, Niwayama S, Hurt J, Byrne JA, Prien S and **Bright RK**. A 2-Dimensional Proteomics-Based Approach to Identify Prostate Tumor Associated Antigens. (in preparation).

Presentations:

- 1) Department of Cell Physiology and Molecular Biophysics, TTUHSC, Seminar Series. **Bright RK**. "Advances in the Active Immunotherapy of Cancer" November 5, 2009
- 2) TTUHSC Cancer Center Grand Rounds. **Bright RK**. "Immuno-Modulation to Enhance Immunity to Tumor Associated Auto-Antigens" November 11, 2009
- 3) Bright JD, Schultz HN, Byrne JA and Bright RK. Inhibition of Tumor Formation in Mice following Vaccination with DNA Encoding a Novel Xenogeneic Tumor Associated Auto-Antigen. Annual Meeting, AACR, Washington DC, April 2010
- 4) Department of Urology, TTUHSC, Grand Rounds. **Bright RK**. "Immuno-Modulation to Enhance Immunity to Over-Expressed Self-Oncoantigens: the TPD52 story." April 30, 2010

Presentation September 2010:

- Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Science, Grand Rounds. Bright RK. "Vaccination Targeting Over-Expressed Self-Oncoantigens: the TPD52 story" September 13, 2010

Degrees completed in year 2:

- Heather N. Schultz successfully defended her master thesis entitled: "Vaccination against the Self-Tumor Associated Antigen Tumor Protein D52 (TPD52)", October 22, 2009. I served as Mrs. Schultz's mentor for her research. Though she was supported by institutional funds her thesis work was directly related to our proposed studies in the TRAMP model and will be included in future progress reports.

Funding applied for in year 2:

- Cancer Prevention Research Institute of Texas (CPRIT); submitted 10/08/09; Robert Bright (PI); "Induction of Immunity to Tumor Associated Auto-Antigens"; total direct costs: \$450,000
- National Cancer Institute; NIH 1 RO1; submitted 06/05/10; Robert Bright (PI); "Cancer Vaccines Targeting Shared Over-Expressed Self-Oncoantigens"; 30% effort; total direct costs: \$1,250,000

Employment:

- Heather N. Schultz, a former student from our lab applied for and obtained a research associate position in the TherapeUTex Pre-clinical Drug Development core at the University of Texas at Austin, July, 2010.

CONCLUSIONS

Overall our efforts to induce tumor immunity following intramuscular TPD52-DNA immunization have been promising. It appears that hD52 may be a more immuno-potent antigen in mice resulting in superior tumor immunity and potent immunologic memory resulting in superior protection from recall tumor challenge compared to DNA vaccination with the murine orthologue of TPD52. We predicted this to be so given the success of others involving the application of murine or rodent tumor associated antigens as xenogeneic vaccines in human clinical trials. We have also begun to evaluate the efficacy of TPD52 protein both hD52 and mD52, as a vaccine either alone or as a heterologous prime-boost approach. We corroborated our previous data demonstrating that Th1-type cellular immunity is critical for TPD52 vaccine induced tumor immunity. We will continue to optimize the potency of DNA-prime with protein-boost in year 3 of this proposal. In addition, we determined that CD25+ Treg cells and TGF- β 1 likely play a critical role in inhibiting TPD52-based immunity following vaccination. This was expected given that TPD52 is a novel tumor associated-auto-antigen. These data are critical for guiding and driving our efforts to optimize vaccination with immune modulation in year 3. Finally, we have uncovered a novel and understudied subset of CD8+ IL-10 secreting regulatory T cells. These CD8 Tregs have been described in models of infectious disease and autoimmunity but have lacked investigation in cancer immunity. Deeper understanding of the role of these novel Treg cells could lead to the development of more potent and effective vaccines targeting over-expressed self-antigens, resulting in more effective immune therapies for many malignancies to include prostate cancer.

RELEVANCE “So What”:

TPD52 is involved in the induction of transformation and metastasis and has been shown by us and other investigators to be over expressed in human prostate, breast, lung, ovarian and colon cancers. Recent studies have identified TPD52 as one of twelve important protein markers, along with MUC-1 and PSA that can be used as a molecular fingerprint of human prostate cancer enabling more accurate and sensitive diagnosis and prognosis of aggressive disease. Our preliminary data suggest that TPD52 vaccination induces immunity capable of rejecting tumors *in vivo* without the induction of autoimmunity or other harmful side effects. We believe the results generated by our research will demonstrate that TPD52 is capable of inducing tumor immunity when administered as a vaccine, and that this immunity will specifically destroy prostate tumor cells.

TPD52-based vaccines defined and characterized by this study could be clinically developed to treat advanced cancer or for preventing progression and metastasis. Recombinant TPD52 protein and/or synthetic peptide(s) could be used to stimulate and expand T cells from patients *ex vivo* for therapeutic transfer back to the patient as adoptive cell therapies (ACT). All these vaccine approaches are relatively easy to generate making the cost much less than other immunotherapy approaches such as dendritic cell (DC)-based vaccines. DNA, protein and peptide vaccines have already proven to have little to no toxicity in patients when studied in clinical trials, making these approaches very safe. Our results are expected to have an important positive impact on cancer vaccine development by providing a deeper understanding of how to induce tumor immunity with vaccines targeting over-expressed self-oncoantigens, together with increased knowledge on how to avoid inducing recognition of antigen positive non-malignant cells, resulting in novel effective prostate cancer vaccine strategies.

REFERENCES

1. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM and Matrisian LM. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res* 15:5323-5337, 2009.
2. Wang R, Xu J, Saramaki O, Visakorpi T, Sutherland WM, Zhou J, Sen B, Lim SD, Mabeesh N, Amin M, Dong JT, Petros JA, Nelson PS, Marshall FF, Zhou He and Chung LW. (2004). *PrLZ*, a novel prostate-specific and androgen-responsive gene of the TPD52 family, amplified in chromosome 8q21.1 and overexpressed in human prostate cancer. *Cancer Res.* 64, 1589–1594.
3. Wang R, Xu J, Mabeesh N, Zhu G, Zhou J, Amin N, He D, Marshall FF, Zhou He and Chung LW. (2007). *PrLZ* is expressed in normal prostate development and in human prostate cancer progression. *Clin. Cancer Res.* 13: 6040-6048.
4. Rubin MA, Varambally S, Beroukhir R, Tomlins SA, Rhodes DR, Paris PL, Hofer MD, Storz-Schweizer M, Kuefer R, Fletcher JA, Hsi BL, Byrne JA, Pienta KJ, Collins C, Sellers WR, and Chinnaiyan AM (2004). Overexpression, Amplification and Androgen-regulation of TPD52 in Prostate Cancer. *Cancer Res.* 64: 3814-3822.
5. Balleine RL, Fejzo MS, Sathasivam P, Basset P, Clarke CL and Byrne JA. (2000). The hD52 (TPD52) gene is a candidate target gene for events resulting in increased 8q21 copy number in human breast carcinoma. *Genes Chromosom Cancer* 29: 48-57.
6. Byrne JA, Tomasetto C, Granier JM, Rouyer N, Mattei MG, Bellocq JP, Rio MC and Basset P. (1995). A screening method to identify genes commonly overexpressed in carcinomas and the identification of a novel complementary DNA sequence. *Cancer Res.* 55: 2896-2903.
7. Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Borresen-Dale AL and Brown PO. (2002). Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc. Natl. Acad. Sci. USA.* 99: 12963-12968.
8. Shehata M, Bieche I, Boutros R, Weidenhofer J, Fanayan S, Spalding L, Zeps N, Byth K, Bright RK, Lidereau R and Byrne JA. (2008). Nonredundant functions for tumor protein D52-like proteins supports specific targeting of TPD52. *Clin Cancer Res.* 14: 5050-5060.
9. Byrne JA, Balleine RL, Schoenberg Fejzo M, Mercieca J, Chiew Y-E, Livnat Y, St. Heaps L, Peters G, Byth K, Karlan BY, Slamon DJ, Harnett P, and deFazio A (2005). Tumor Protein D52 (TPD52) is overexpressed and a gene amplification target in ovarian cancer. *Int. J. Cancer* 117: 1049-1054.
10. Bismar TA, Demichelis F, Riva A, Kim R, Varambally S, He L, Kutok J, Aster JC, Tang J, Kuefer R, Hofer MD, Febbo PG, Chinnaiyan AM and Rubin MA. (2006). Defining aggressive prostate cancer using a 12-gene model. *Neoplasia* 8: 59-68.
11. Byrne JA, Mattei MG and Basset P. (1996) Definition of the tumor protein D52 (TPD52) gene family through cloning of D52 homologues in human (hD53) and mouse (mD52). *Genomics* 35:523-532.
12. Lewis JD, Payton LA, Whitford JG, Byrne JA, Smith DI, Yang L and Bright RK. (2007) Induction of tumorigenesis and metastasis by the murine orthologue of tumor protein D52. *Mol Cancer Res* 5:133-144.
13. Payton LA, Lewis JD, Byrne JA and Bright RK. (2008) Vaccination with metastasis-related tumor associated antigen TPD52 and CpG/ODN induces protective tumor immunity. *Cancer Immunol Immunother* 57:799-811.
14. Lewis JD, Sullivan LA, Byrne JA, de Riese W and Bright RK. Memory and cellular immunity induced by a DNA vaccine encoding self-antigen TPD52 administered with soluble GM-CSF. *Cancer Immunol Immunother* 58:1337-1349, 2009.
15. Mirshahidi S, Kramer VG, Whitney JB, Essono S, Lee S, Dranoff G, Anderson KS and Ruprecht RM. Overlapping synthetic peptides encoding TPD52 as breast cancer vaccine in mice: prolonged survival. *Vaccine* 27:1825-1833, 2009.